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Quantitation of infarct size in man by means of plasma enzyme levels[1]

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A method is described in which the extent of myocardial infarction in man is assessed by mathematical analysis of the rise in plasma enzyme levels after infarction. Five enzymes are used in this study: lactate dehydrogenase (LDH); alpha-hydroxybutyrate dehydrogenase (α-HBDH); aspartate aminotransferase (GOT); creatine phosphokinase (CPK); and phosphohexoseisomerase (PHI).

It is shown that a reasonable assessment of the total enzyme release, reflecting the extent of the infarcted area, can be made when a sufficient number of blood samples are taken after infarction. This could provide a method by which to judge therapeutic effects of intervention in the course of a myocardial infarction, as demonstrated in this study by the assessment of the effect of urokinase on the enzyme release after an infarct.

The importance of the determination of serum enzyme levels in patients suspected of an acute myocardial infarction has been well established. Extensive studies have been reported on the clinical usefulness of estimation of serum levels of several enzymes among which GOT, LDH, α-HBDH, and, somewhat more recently, CPK, are used most widely (Dreyfus *et al.*, 1960; Elliott and Wilkinson, 1961; Konttinen and Halonen, 1962; LaDue, Wroblewski, and Karmen, 1954; Wroblewski, 1957; Agress *et al.*, 1955).

However, these studies were mainly of a qualitative nature, used for confirming a diagnosis or for differentiation from other diseases. With the introduction of coronary care units death from myocardial infarction caused by arrhythmias has decreased but power failure still remains an important complication of myocardial infarction. It has been shown that cardiogenic shock after infarction is correlated with large infarcted areas (Harnarayan *et al.*, 1970).

As more studies become available which show that the extent of the infarcted area in experimental animals can be modified by certain interventions (Ginks *et al.*, 1972; Libby *et al.*, 1973; Maroko *et al.*, 1971, 1972; Roberts, 1974; Sybers *et al.*, 1973), a quantitative assessment of myocardial infarction in man becomes more and more important as it provides a tool with which the effect of therapeutic regimens in man can be judged.

The concept of using the plasma enzyme increases for quantitative analysis in acute myocardial infarction, as was introduced by our group some years ago (Witteveen, Hermens, and Hemker, 1970; Witteveen *et al.*, 1971), has been developed further and is applied in this study to a group of 15 patients.

The clinical usefulness of the method is shown by comparing a group of 5 patients, who received urokinase, a thrombolytic agent, with a control group of 10 patients.

Subjects and methods

Patients

Plasma enzyme levels were measured, and used as a basis for further calculations, in 15 patients with proven myocardial infarction. The diagnosis of acute myocardial infarction was considered certain when the clinical picture was typical and the electrocardiogram showed definite development of an infarction pattern. The group comprised 12 men and 3 women with ages ranging from 41 to 76 years. Details are given in Table 1. The electrocardiogram was monitored as the clinical situation required. The standard treatment consisted of bedrest, oxygen (usually 4 l/min by nasal catheter) and salt and fluid restriction during the first few days. Pain was suppressed by opiates, usually 5 mg nicomorphine,

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TABLE I *Data on the patients in the present series*

Case No.	*Age (yr)*	*Sex*	*Site of infarction*	*Admission time after first severe symptoms (hr)*	*Max. temperature in initial period after infarction (°C)*	*Major complications*
1	43	M	Anterior wall	1	38.6	Adams-Stokes attacks and short episodes of complete atrioventricular block 36 hours after admission
2	73	F	Inferior wall	3½	38.9	Ventricular fibrillation
3	41	M	Inferior wall	8	38.1	None
4	42	M	Anterior wall	1½	39.2	None
5	72	F	Inferior wall	13	38.8	Urinary tract infection
6	76	M	Inferior wall	10	39.0	Pulmonary congestion requiring diuretics
7	69	M	Inferior and lateral walls	6	38.9 (after 44 hr) 39.6 (after 70 hr)	Paroxysmal atrial fibrillation starting 4 days after admission; slight mitral regurgitation
8	48	M	Inferior wall	6	38.0	None
9	55	M	Anterior wall	1¼	38.8	None
10	57	M	Anterior wall	4	38.4	None
11	54	M	Inferior and lateral walls	2	38.5	None
12	59	M	Inferior wall	1½	37.8	None
13	47	M	Anteroseptal	2	38.5	None
14	50	F	Subendocardial, anteroseptal	5	38.3	None
15	59	M	Anteroseptal	1½	38.2	None

combined with 0.25 mg atropine, injected intravenously. All patients were sedated with diazepam, 5 mg given three times daily. If ventricular premature beats developed with a frequency of more than 5 per minute, lignocaine was given in a dosage of 2 mg per minute. If signs of pulmonary congestion were found on *x*-ray or physical examination the diuretic frusemide was given. Patients with cardiogenic shock or severe heart failure were not included in the investigation.

Anticoagulant therapy was applied in all cases. All patients were given phenprocoumon in the required dosage except Cases 1, 2, 10, 11, and 12, who received warfarin sodium. The latter group was also given urokinase,[1] a thrombolytic agent. This drug was administered as soon after admission as possible in an initial loading dose of 7200 CTA units/kg body weight, given over an interval of 10 minutes, after which an infusion of 3600 CTA units/kg body weight per hour was given for 18 hours.

Enzyme determinations

When a patient was admitted to the hospital with a myocardial infarct blood was drawn as soon as possible and thereafter every 4 hours for 2 or 3 days. After that progressively fewer samples were taken until plasma enzyme levels had returned to normal. Each sample consisted of 10 ml venous blood collected into tubes containing 0.2 ml 20 per cent sodium citrate solution to prevent clotting. After careful mixing of the blood with the citrate, centrifugation was done at 900 *g* for 10 minutes to remove erythrocytes and leucocytes and subsequently at 40 000 *g* for 20 minutes to remove thrombocytes. Thereafter the sample was stored at −20°C until determinations were carried out.

Heart tissue was obtained at thoracic surgery on other patients for determination of the enzyme activity in the normal myocardium. Immediately after removal the tissue was placed in isotonic saline at 0°C, weighed, and homogenized in a Turrax homogenizer as soon as possible, usually within 10 to 15 minutes. After sedimentation of any remaining particles the enzyme activity in the supernatant fluid was determined.

The enzymes determined in this study were aspartate aminotransferase (GOT, E.C.[1] 2.6.1.1.), lactate dehydrogenase (LDH, E.C. 1.1.1.27.), alpha-hydroxybutyrate dehydrogenase (α-HBDH, E.C. 1.1.1.27), creatine phosphokinase (CPK E.C. 2.7.3.2.), and phosphohexoseisomerase (PHI, E.C. 5.3.1.9.). All enzyme determinations were done spectrophotometrically, at 25°C, measuring either the appearance or disappearance of NADH or NADPH. For GOT, LDH, and α-HBDH, commercially available test kits (Boehringer,

[1] Kindly supplied by Hoffman-La Roche, Basle, Switzerland.

[1] Enzyme Code (Dixon and Webb).

Duration in hosp. (wk)	*Special treatment*	*Comments*	*Weight (kg)*	*Haemato-crit (%)*	*Calculated plasma vol. (l)*
3	External pacemaker on demand; urokinase started 5½ hr after infarction	None	75	46	2.8
5	Urokinase started 8 hr after infarction	None	60	37	2.6
4½	None	Diabetes mellitus for 15 yr	70	45	2.7
4	None	Hypertension known for 6 yr	90	43	3.6
4	None	None	55	42	2.2
4	None	Anterior inf. at age 71, cerebrovasc. episode at age 74	70	51	2.4
6	Digoxin, diuretics	Long period of pyrexia; high E.S.R. (105 mm); cardiac enlargement; pleural effusion; no antibodies against myocardium found	75	44	2.9
4	—	None	74	48	2.7
3½	—	None	68	46	2.6
3½	Urokinase started 5 hr after infarction	None	74	44	2.9
4	Urokinase started 3¼ hr after infarction	High blood pressure for several yr	69	41	2.6
3½	Urokinase started 5¼ hr after infarction	None	62	44	2.4
4	—	None	64.3	43	2.6
4	—	None	71	45	2.7
4	—	Gastric ulcer in 1955; 3 wk before admission anginal pain (not severe)	63	44	2.5

type TC-A-I, TC-G-I and TC-HD) were used. CPK was determined according to Rosalki (1967) and PHI according to Weber and Wegmann (1968). Enzyme activities were expressed in IU/litre, in which one IU of enzyme catalyses the change of one micromole substrate per minute.

Mathematical analysis of data

Principle After the cessation of blood flow to an area of the myocardium, the tissue in this area dies and releases its enzymes. This release results in a sudden increase in plasma enzyme levels, followed by a gradual decrease to normal values because of diffusion and elimination. If no diffusion occurred from the plasma to an extravascular space, i.e. if the distribution volume equalled the plasma volume, the change in the total amount of enzyme in the plasma at each moment would be a function of influx from the infarcted tissue and of elimination caused by normal biological degradation:

$$V_1\frac{dC_1}{dt} = F(t) - k.V_1C_1 \qquad (1)$$

in which V_1 is the plasma volume, C_1 the concentration of enzyme in the plasma, F(t) a function, describing the rate of appearance of enzyme in the plasma, and k the disappearance constant of the enzyme from the plasma.

Once the enzyme release from the infarcted area has stopped, $F(t)=0$ and the formula becomes

$$\frac{dC_1}{dt} = -k.C_1(t) \qquad (2)$$

This equation has as its solution:

$$C_1 = C_0.e^{-k.t} \qquad (3)$$

showing an exponential decay of enzyme.

Theoretically, by plotting the measured enzyme levels against time on a semilogarithmic scale, starting at a point at which one can be sure that the release of enzyme has stopped (approximately 40 hours after the infarction), it should be possible to determine the elimination constant k. From the curve in which C_1 is plotted against time, dC_1/dt could be obtained for the period of enzyme release and with k known, the release F(t) could be derived from formula (1).

Another factor – diffusion into an extravascular space – has to be taken into account. It is known for enzymes as well as for other proteins (Dunn, Martins, and Reissmann, 1958; Schultze and Heremans, 1966) that the protein is not confined to the plasma but that diffusion takes place over a larger volume, representing the plasma volume V_1 plus at least one extravascular volume V_e. Together these can be viewed as the real distribution volume. The rate of diffusion is proportional to the difference in concentration between the intra- and extra-

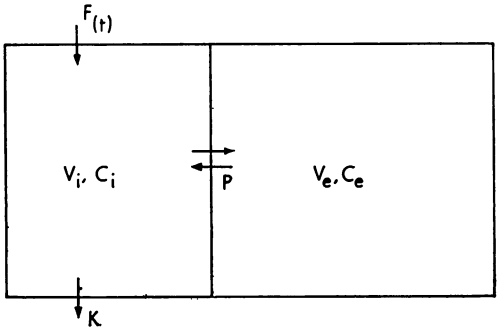

FIG. 1 *Model representing V_i = intravascular volume; V_e = extravascular volume; C_i = intravascular enzyme activity in IU/l; C_e = extravascular enzyme activity in IU/l; $F(t)$ = amount of enzyme (IU) entering the intravascular volume per hour; k = rate constant of elimination of the enzyme from the plasma (hours)$^{-1}$; and P = permeability constant.*

vascular volumes and to a permeability constant P (Fig. 1).

The balance equations for the exchange of enzyme in this dual compartment model are given by the following equations:

$$V_i \cdot \frac{dC_i}{dt} = -k\ V_iC_i + P(C_e - C_i) + F(t) \qquad (4)$$

$$V_e \cdot \frac{dC_e}{dt} = P(C_i - C_e) \qquad (5)$$

Using these formulae one can in any given patient calculate F(t), the amount of enzyme released per hour. To make this possible, however, V_e and P have to be known. By more elaborate mathematical analysis of the data (Witteveen, 1972; Hermens *et al.*, 1975) we obtained the following values for P/V_i and V_e/V_i:

$$P/V_i = 0.06 \pm 0.04 \text{ (hours)}^{-1}$$

$$V_e/V_i = 0.3 \pm 0.2 \qquad (6)$$

As indicated, there is a considerable variation in these values. However, a 100 per cent variation in P/V_i has hardly any effect on the calculated amount of total enzyme release, whereas a 100 per cent variation in V_e/V_i changes this calculated amount by approximately 20 per cent.

To calculate the values in each individual patient a computer programme was written in which the above-mentioned formulae and values were used. In this way the amount of enzyme released from the infarcted area into the circulation could be obtained. After multiplication of this quantity of enzyme released per litre(Q) by the plasma volume (V_i), obtained from body weight and haematocrit, an equivalent amount of infarcted heart tissue (A) is calculated as follows. From *in vitro* experiments (Witteveen, 1972; Hermens, to be published), it is known that anoxic human heart tissue only loses 80 per cent of its LDH content into the circulation. This means that ($Q \times V_i$) has to be multiplied by a factor 1.25 to obtain the minimal amount of heart tissue that is infarcted. When ($Q \times V_i \times 1.25$) is divided by 128 which is the enzyme content in IU per g normal heart tissue (Table 2), the equivalent amount in grams of infarcted heart tissue is obtained. For the other enzymes in the study the corresponding value of Q was first transformed into the equivalent value of Q_{LDH}, using best fit equations (Fig. 2), after which an analogous procedure was followed. LDH was chosen as reference enzyme because it is eliminated slowly and is less sensitive to irregularities in the release.

TABLE 2 *Human heart tissue enzyme activity; means of 15 experiments*[1]

	LDH	*α-HBDH*	*GOT*	*CPK*	*PHI*
Mean	128	101	96	370	143
SD (%)	12	11	19	10	10

[1] Enzyme activity expressed in IU/g wet tissue.

Results

Table 2 gives the values for the enzyme activity measured in human myocardial tissue, obtained at cardiac surgery. Table 3 gives the characteristics of enzyme levels measured in patients: maximal value,

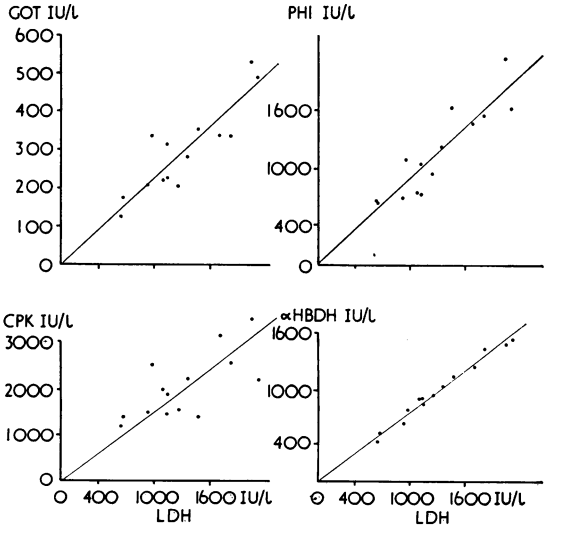

FIG. 2 *Correlations between LDH and the other enzymes. Best fit straight line through the origin for α-HBDH; $y = 0.74x$; GOT: $y = 0.22x$; CPK: $y = 1.50x$; PHI: $y = 0.89x$.*

TABLE 3 *Maximal plasma activity, time of maximal activity, and half-life time in plasma of 5 enzymes under study*

Case No.	*LDH*			*α-HBDH*			*GOT*			*CPK*			*PHI*		
	a	*b*	*c*	*a*	*b*	*c*	*a*	*b*	*c*	*a*	*b*	*c*	*a*	*b*	*c*
1*	1785	23.0	51	1259	23.0	61	267	17.2	19	1290	13.2	13	763	13.2	7
2*	1430	34.5	48	1043	34.5	45	270	20.0	21	1485	20.0	20	833	20.0	21
3	1087	47.0	39	839	52.5	39	129	33.2	20	1007	33.2	18	311	28.8	14
4	875	30.0	44	620	35.0	42	97	18.1	19	838	18.1	15	254	15.0	8
5	1265	45.0	68	955	45.0	60	128	33.2	29	1009	28.9	21	306	28.9	24
6	984	38.5	70	693	42.7	66	174	30.5	26	576	22.5	20	315	26.5	20
7	780	41.4	48	626	41.4	47	123	28.0	22	690	24.0	14	249	20.0	17
8	940	35.7	48	672	35.7	51	121	27.2	17	759	23.1	15	256	23.1	11
9	822	32.4	66	710	32.4	55	115	28.1	17	975	20.2	18	191	20.2	13
10*	837	19.8	38	655	19.8	43	133	19.8	15	994	16.5	15	418	16.5	9
11*	959	21.3	77	664	21.3	85	150	15.0	22	1265	15.0	13	428	15.0	9
12*	756	30.1	62	498	30.1	67	94	26.2	22	741	22.0	13	215	19.2	12
13	536	31.7	42	419	31.7	42	71	24.0	18	529	20.0	13	174	20.0	17
14	554	43.7	53	395	43.7	53	70	31.0	19	468	25.3	18	139	25.3	15
15	130	31.3	—	72	31.3	—	15	22.3	—	150	14.0	—	42	14.0	—

a = maximal plasma enzyme activity in IU/litre.
b = time of maximal plasma activity in hours after infarction.
c = overall half-life time measured from the time of maximal plasma activity, mean ± SD for LDH 54 ± 12; α-HBDH 54 ± 13; GOT 20 ± 4; CPK 16 ± 3; PHI 14 ± 5 hours.
* Patients received urokinase.

time of maximal value in hours after the infarction, and the time in which half maximal levels were reached calculated from plasma enzyme activity.

Table 4 shows the amounts of enzyme released into one litre of plasma (Q) and the elimination constants k; from this, the equivalent amount of heart tissue in grams (A) is obtained as described in the methods. For α-HBDH, GOT, CPK, and PHI the correction factors as derived from Fig. 2 were used.

Fig. 3 to 7 give representative curves for one of the patients (Case 8). In these figures, curve

TABLE 4 *Elimination constant, enzyme release, and calculated infarct size*

Case No.	*LDH*			*α-HBDH*			*GOT*			*CPK*			*PHI*		
	k	*Q*	*A*	*k*	*Q*	*A*	*k*	*Q*	*A*	*k*	*Q*	*A*	*k*	*Q*	*A*
1*	0.0126	2113	58	0.0125	1533	55	0.0576	485	60	0.0849	2205	40	0.1803	1624	50
2*	0.0134	2042	52	0.0113	1483	50	0.0576	523	60	0.0705	3473	59	0.0759	2137	61
3	0.0157	1699	45	0.0164	1240	43	0.0513	334	40	0.0736	3123	55	0.1652	1463	43
4	0.0194	1253	44	0.0202	957	44	0.0742	204	33	0.0602	1559	36	0.2903	948	37
5	0.0131	1819	39	0.0133	1423	40	0.0546	334	33	0.0661	2562	37	0.1536	1548	37
6	0.0139	1469	34	0.0167	1145	35	0.0405	353	38	0.0684	1402	22	0.1577	1630	43
7	0.0182	1131	32	0.0181	917	34	0.0670	315	41	0.0711	1468	28	0.1714	1050	33
8	0.0157	1355	36	0.0189	1038	36	0.0715	280	34	0.1037	2217	39	0.2436	1236	37
9	0.0129	1090	38	0.0134	906	30	0.0478	221	26	0.0615	1998	34	0.1625	748	21
10*	0.0102	969	27	0.0130	785	29	0.1321	335	43	0.1127	2505	47	0.1714	1095	35
11*	0.0082	1134	29	0.0086	855	29	0.0428	225	26	0.0558	1895	32	0.1054	730	21
12*	0.0135	928	22	0.0122	634	20	0.0580	207	22	0.0662	1498	23	0.1611	697	18
13	0.0274	664	17	0.0243	535	18	0.0781	176	20	0.0692	1396	24	0.1675	648	18
14	0.0124	639	17	0.0121	442	15	0.0439	127	15	0.0754	1193	21	0.1890	665	20
15	—	—	—	—	—	—	—	—	—	0.0413	255	4	—	—	—
Mean ± SD	0.0148 ± 0.0046			0.0151 ± 0.0042			0.0626 ± 0.0232			0.0720 ± 0.0177			0.1711 ± 0.0511		

Q = total enzyme release in IU per litre vascular volume.
k = elimination constant.
A = equivalent amount of heart tissue in grams (see text).
* Patients treated with urokinase.

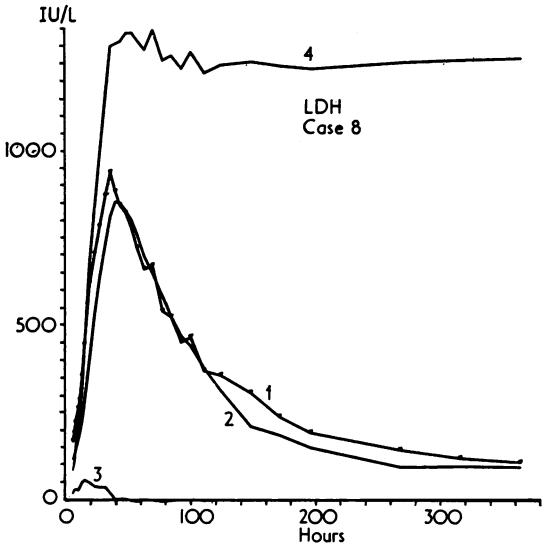

FIG. 3 *LDH values for Case 8. 1 = measured plasma enzyme activity; 2 = calculated extravascular enzyme activity; 3-calculated enzyme release in IU/l per hr; 4 = calculated total enzyme release.*

1 represents the plasma enzyme activity measured in these patients at regular intervals after the infarction. Curve 2 gives the calculated extravascular activity C_e in IU per litre extravascular volume. The calculations were based on the estimations of extravascular volumes and of diffusion constants

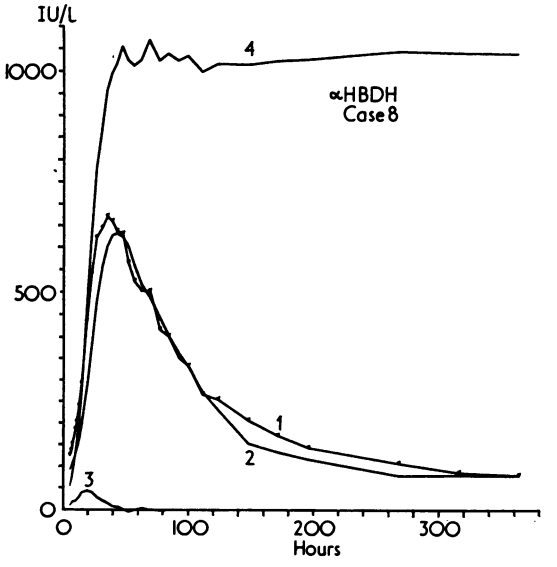

FIG. 4 *Same values as in Fig. 3 for α-HBDH.*

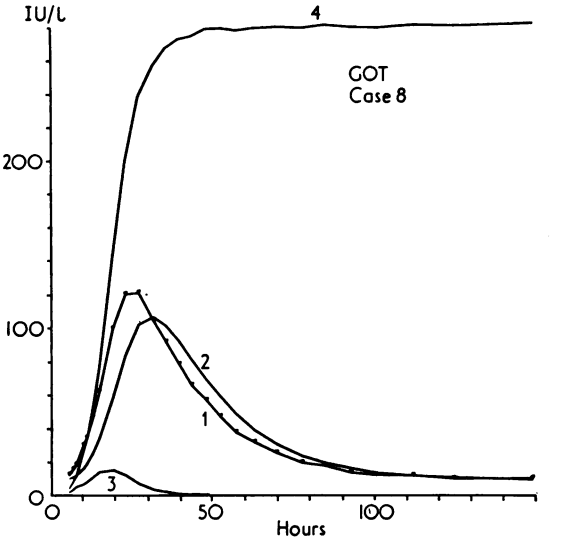

FIG. 5 *Same values as in Fig. 3 for GOT.*

TABLE 5 *Time of maximal plasma enzyme activity*

	LDH	*α-HBDH*	*GOT*	*CPK*	*PHI*
a	37.7 (SD = 6.3)	39.1 (SD = 7.0)	27.6 (SD = 4.9)	22.9 (SD = 5.4)	22.2 (SD = 5.3)
b	25.7 (SD = 6.3)	25.7 (SD = 6.3)	19.6 (SD = 4.2)	17.3 (SD = 3.6)	16.8 (SD = 2.8)

a = mean value for control patients (n = 10).
b = mean value for patients treated with urokinase (n = 5).

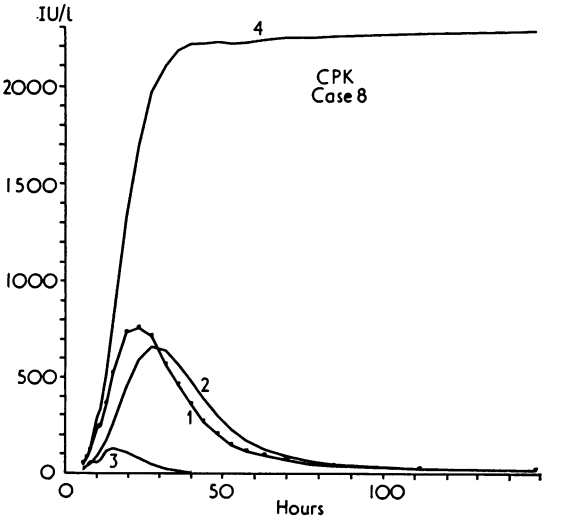

FIG. 6 *Same values in Fig. 3 for CPK.*

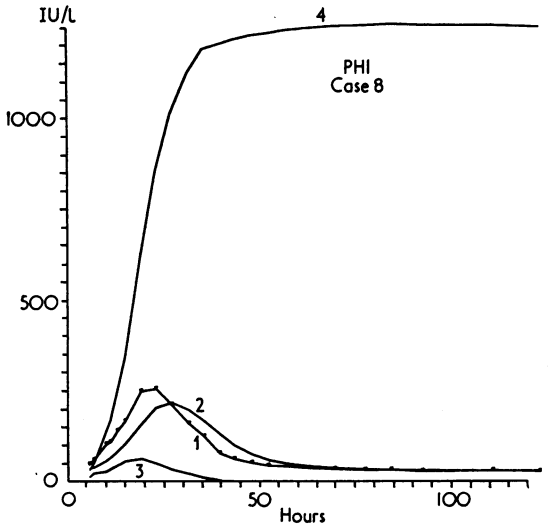

FIG. 7 *Same values as in Fig. 3 for PHI.*

as described in the methods. Curve 3 shows the rate at which the enzymes were released into the circulation from the necrotic tissue, expressed in IU/litre per hour. Curve 4 shows the total amount of enzyme released into one litre of plasma. The final value in this curve (obtained at 48 hours) is given in Table 4 under column Q.

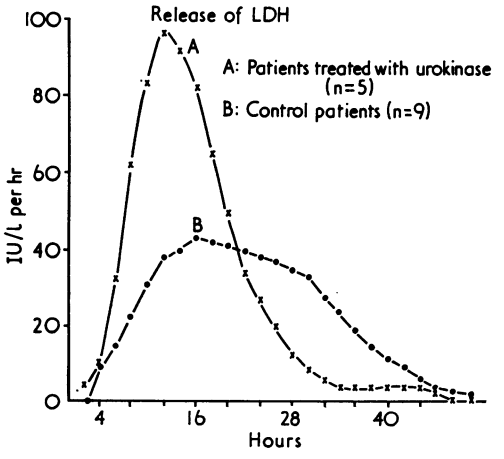

FIG. 8 *Average release function F(t) for LDH, showing shortening of enzyme release phase in urokinase-treated patients. The curve represents averages obtained from infarcts ranging in size from 17 to 58 g destroyed tissue (see Table 4).*

Discussion

As can be seen from Table 3, a wide range of plasma enzyme maxima was found. A maximal plasma level is obtained when enzyme breakdown equals enzyme release. This explains the fact that the time needed to reach a maximal plasma level is proportional to the height of the maximum.

LDH and α-HBDH reach the maximal value later than GOT, PHI, and CPK, i.e. about 36 and 24 hours after the infarction, respectively. This is mainly because of the much higher rate at which GOT, PHI, and CPK disappear from the circulation, as indicated by the disappearance constants which were up to 10 times higher than the values for LDH and α-HBDH. For the latter enzymes, definite rises were often found for more than 2 weeks after the acute attack.

It is clear from our findings that the leakage of enzymes from the cells starts early after the infarction. All patients admitted soon after the onset of acute symptoms already showed some enzyme release 5 hours after the infarction. This holds for all enzymes investigated in this study. From that time on, the amount of enzyme coming into the circulation rose rapidly to reach a maximum between 10 and 20 hours after the moment of infarction (Fig. 8).

The fact that the rate of release (curve 3, Fig. 3 to 7) rises progressively after the initial period and reaches a maximum after 10 to 20 hours reflects the increase in cell membrane permeability resulting from the loss of normal membrane function. As time goes on, the loss of normal membrane structure permits a more rapid diffusion of larger molecules like those forming the enzymes.

Another factor that might explain the low rate of release in the initial phase and the finding of a later maximum is probably that not all cells die and disintegrate at the same moment. Present research in our laboratory (Hermens *et al.*, 1975) indicates that the necrotic process is self-generating in its initial phase. This can be explained by the hypothesis that the cell contents of a disintegrating cell enhance the lysis of neighbouring cells. The decline in the release rate and the finding that no further loss of enzymes occurs after about 48 hours implies that by then the cells have lost their enzymes more or less completely. A remarkable phenomenon was found in patients treated with the thrombolytic agent urokinase. As a group, these patients had an earlier maximal plasma enzyme level, as is shown in Table 5. The earlier maximum found for the urokinase-treated patients might be explained in two ways. First, the breakdown of the enzymes in the circulation could be much more rapid under the

influence of the thrombolytic agent. That this is not the case is shown by the disappearance constants (Table 4) which have a wide range but are not obviously different in the urokinase-treated group. A second possibility is a more rapid washout of the enzymes from the infarcted area under the influence of urokinase. If we consider the time at which the plasma level is maximal (Table 6) and allocate patients in two treatment groups (with and without urokinase), there is a very clear difference which is statistically significant at a 0.01 level for LDH, α-HBDH, and GOT and at a 0.05 level for CPK and PHI (Wilcoxon, one-sided).

For LDH, for example, the maximum lies at 37.5 hours (mean) for the 10 untreated patients and at 25.7 hours for the urokinase-treated patients, which is consistent with a more rapid loss of the contents of necrotic cells. The same phenomenon is shown in Fig. 8, in which the release rate of LDH in IU/l per hr is compared for the two groups. The more rapid release of enzymes in the urokinase-treated group might be explained by a thrombolytic effect of urokinase: it restores patency or prevents further occlusion in vessels, for example collaterals supplying the peripheral zone of the infarcted area. It is conceivable, too, that a primary thrombus responsible for the infarction is also dissolved to some extent by the same process. From our data we cannot conclude that the total amount of necrotic tissue is diminished. Clinical trials of thrombolytic agents did not show any significant difference in mortality between treated and untreated groups (Brogden, Speight, and Avery, 1973; Burkart *et al.*, 1973).

An unknown factor in the calculations of infarct size from plasma enzyme levels in humans is the quantity of enzymes that is destroyed or inactivated locally and thus cannot be released into the circulation. We account for the amount of enzyme that is not released in *in vitro* experiments (20% for LDH, Witteveen, 1972), but an overall underestimation might be possible because of local *in vivo* breakdown.

Shell, Kjekshus, and Sobel (1971) and Shell *et al.* (1973) developed a model for infarct quantitation based upon experiments with dogs. Their model assumes that the distribution space for CPK forms 11.4 per cent of body weight, which implies a ratio $V_e/V_i = 2.5$. Such a large extravascular pool is not in accordance with our results (cf (6)). Though their underlying model does not seem realistic to us, their calculated infarct sizes are probably not overestimated, as use was made of an experimentally tested correlation in dogs.

The fact that there is still considerable uncertainty about the human values for the parameters V_e/V_i and P/V_i is because a clear-cut biphasic disappearance of enzyme activity from the plasma (as predicted by equations (4) and (5)) would only be observed with instantaneous injection of activity at $t=0$. In that case the fast and the slow phase of the disappearance could be measured with more precision, and the uncertainty in the parameters V_e/V_i and P/V_i would be correspondingly reduced. Such experiments with injections of enzyme activity have been done with dogs (Dunn *et al.*, 1958; Shell *et al.*, 1971) but, as discussed in this paper, those results cannot be extrapolated to man.

One obvious difference between experimental material obtained from animal experiments and data obtained from humans is the value of the metabolic clearance constant for CPK which in the experiments of Shell *et al.* is very high and remarkably constant (0.0048 ± 0.0003 min^{-1}; mean $\pm$ SD), while as can be seen from Table 4, the k value for CPK as well as for the other enzymes shows much more variation in patients. This implies that in human studies the clearance constant k has to be determined from the data obtained and used for further calculations in each particular case obviating spuriously high or low estimate of infarct size that would ensue if some average fixed value for k had been used.

Taking these limitations in mind, however, and accounting for the distribution volumes, reasonable estimates of infarct size can be made in humans, which can be correlated for example with cardiac function (Mathey *et al.*, 1974). This also makes it possible to evaluate the effect of certain therapeutic interventions, like the treatment with urokinase in this study.

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